EXHIBIT 18

Amendment in Response to
November 3, 2008 Office Action
Submitted: May 4, 2009

Serial No. 10/821,726

Filed: April 8, 2004

Applicants: Michael Wayne Graham et al.

named after the place of its invention in Eugene, Oregon. The model is a set of three partial differential equations that describe the reaction–diffusion process. Showalter and colleagues added a term to account for the photosensitive generation of bromide ions, and predict wave propagation patterns remarkably similar to those observed in the experiment. Before this experiment, STSR had been studied only theoretically or by numerical or electronic simulation in one-dimensional sets of coupled[6,7] and uncoupled[8] elements, and in two-dimensional arrays of threshold elements[9]. But those 2D simulations, in spite of their simplicity, mimic all the features of the present experiment.

The implications of the present experiment extend far beyond chemical dynamics. Spiral waves, spontaneously generated by noise, have also been simulated with the Oregonator (Fig. 1b). They are strikingly similar to recent observations of noise-initiated and sustained long-range coherent waves of calcium ions in cultured brain tissue[10] (Fig. 1c) indicating a similar underlying dynamical process. The possibility that calcium waves transmit or coordinate information over centimetre distances in glial cell networks (that is, in the brain) has already been suggested, but the role of noise remained obscure. Now that noise-sustained spiral waves have been observed in a well characterized chemical system, we can speculate that spatiotemporal noise may be an important feature of the brain's working. □

Frank Moss is at the Center for Neurodynamics, University of Missouri at St Louis, St Louis, Missouri 63121, USA.

e-mail: mossf@umslvma.umsl.edu

1. Kádár, S., Wang, J. & Showalter, K. *Nature* 391, 770–772 (1998).
2. Winfree, A. T. *Science* 175, 634–636 (1972).
3. Wiesenfeld, K. & Moss, F. *Nature* 373, 33 (1995).
4. Gammaitoni, L., Hanggi, P., Jung, P. & Marchesoni, F. *Rev. Mod. Phys.* 70, 223–288 (1998).
5. Field, R. J. & Noyes, R. M. *J. Chem. Phys.* 60, 1877–1884 (1974).
6. Lindner, J. F. *et al. Phys. Rev. Lett.* 75, 3–6 (1995).
7. Löcher, M., Johnson, G. A. & Hunt, E. R. *Phys. Rev. Lett.* 77, 4698–4701 (1996).
8. Collins, J. J., Chow, C. C. & Imhoff, T. T. *Nature* 376, 236–238 (1995).
9. Jung, P. & Mayer-Kress, G. *Phys. Rev. Lett.* 62, 2682–2686 (1995).
10. Jung, P., Cornell-Bell, A., Shaver Madden, K. & Moss, F. *J. Neurophysiol.* (in the press).

Functional genomics

Double-stranded RNA poses puzzle

Richard W. Wagner and Lin Sun

The human genome is predicted to contain between 50,000 and 100,000 genes[1]. To work out what these genes do, an array of techniques is needed to evaluate the protein–protein interactions and biochemical pathways of any gene product. The nematode worm *Caenorhabditis elegans* is an excellent system for such studies because of its well-understood genetics and development, evolutionary conservation to human genes, small genome size and relatively short life cycle. The 100-megabase-pair genome will be completely sequenced this year, and a total of 17,000 genes have been predicted, many with human counterparts. Approaches used to manipulate gene expression in *C. elegans* include transposon-mediated deletion[2], antisense inhibition[3] and direct isolation of deletions after mutagenesis[4,5]. Although these methods have proved useful, limitations still exist.

On page 806 of this issue, Fire and colleagues[6] describe a remarkable and surprising technique for inhibiting gene function in *C. elegans*. They turned off a specific gene in progeny worms by microinjecting double-stranded RNA (dsRNA) complementary to the coding region of the gene into the gonads of adult animals. Using a well-characterized gene, *unc-22*, which encodes a non-essential myofilament protein, they showed that injection of dsRNA produced a phenotype characteristic of *unc-22* inhibition — twitching.

In a series of well-controlled studies, the authors also found that injection of dsRNA targeted to a reporter gene for green fluorescent protein resulted in a dramatic — and specific — decrease in protein production. Furthermore, when they injected dsRNA targeted to another gene, *mex-3*, the result was a loss of *mex-3* RNA in early-stage embryos. In other words, at the levels of phenotype, RNA and protein, the interference with gene expression was specific and reproducible.

Perhaps most astounding is the phenomenon that the dsRNA causes gene inhibition. Previously[3], Fire and co-workers had been puzzled by the fact that antisense RNA alone — which is often used to inactivate sense messenger RNA — was only marginally effective. Furthermore, results using the antisense RNA were mimicked by injection of sense RNA, a control in their studies. They later found out that these data could be largely explained by an artefact of the transcription process that was used to generate the antisense and sense RNAs; namely, dsRNA fragments.

Additional experiments by Fire *et al.*, designed to shed light on the possible mechanism of the dsRNA-mediated inhibition, painted an even more mystifying picture. For example, even when only a few copies of the dsRNAs are present in each cell, they are active against highly abundant RNAs. This indicates that the interference occurs either by a catalytic mechanism or at the chromosomal level — and not by a conventional antisense mechanism. The authors also found that only dsRNAs that are complementary to coding regions of the gene are active, and not, for example, those targeted to introns or promoter regions. This argues against a generalized mechanism involving chromosomal inactivation, such as chromosomal deletion. Moreover, dsRNA interference seems to cross cellular boundaries with

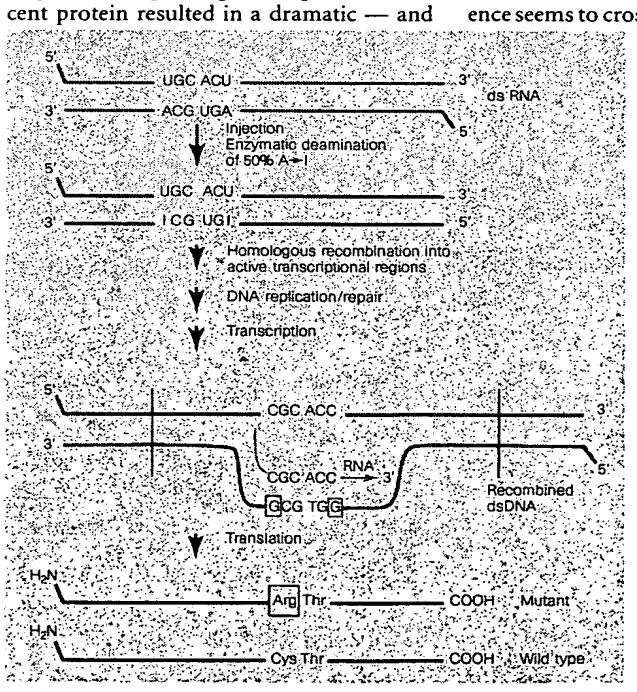

Figure 1 Possible mechanism for inhibition of gene expression in *C. elegans* by double-stranded RNA. Fire *et al.*[6] have convincingly shown that, at the phenotype, RNA and protein levels, dsRNA-mediated interference with gene expression is specific and reproducible. Perhaps, on injection into worms, dsRNA is modified by dsRNA adenosine deaminase. Transfer of this information back into the chromosome may occur by a recombination event. After replication and mismatch repair, transcription and translation result in mutant proteins that have impaired function.

ease. Gene inhibition was observed in progeny when dsRNA was injected into the body cavity of the adult (gonadal injections had been thought to be necessary), and in somatic tissues of young adults after injection into their body cavity.

What kind of mechanism have Fire and colleagues uncovered? This is not the first puzzle posed by dsRNA. Almost ten years ago, Bass and Weintraub[7] and Wagner *et al.*[8] discovered an enzyme that binds dsRNA and deaminates adenosines in the duplex to inosines. After a feverish hunt for the cellular function of the dsRNA adenosine deaminase, it was found to be involved in the post-transcriptional editing of messages. Inosines are read by the cellular machinery as guanosine, so the enzyme could alter the genetic make-up of mRNA (reviewed in refs 9, 10).

Could this dsRNA adenosine deaminase be involved in a complicated pathway that results in gene inhibition in *C. elegans*? Quite possibly. The enzymatic activity has been found in *C. elegans*, and would probably treat the injected dsRNA as a substrate. A specialized homologous recombination system would be needed, which would use the modified dsRNA to transfer the genetic alterations into the chromosome (Fig. 1).

This model fits some of the data: modification of adenosines to inosines alters the genetic make-up of the injected dsRNA; transfer of this information into the genome by recombination would affect coding (but not intronic) regions; and mutations introduced by the inosine substitutions would affect the ability to detect mRNA and, at least partially, the function of the protein. These mutations could account for the surprising result that only a few copies of dsRNA are required per cell, because they would have an effect at the level of the chromosome. Of course, such a model is a stretch of the imagination and is not supported by all of the data. For example, attempts to use homologous recombination with dsDNA in *C. elegans* have largely failed[3].

Fire and colleagues[6] have uncovered a complex and intriguing mode of regulation in *C. elegans*. Does dsRNA perform a biological function in *C. elegans* (and is this function titrated out by the microinjected dsRNA)? Does a similar phenomenon exist in other organisms? What would happen if transgenic animals or plants were generated expressing both the sense and antisense strands of a transgene? A similar mode of action would not be suspected to occur in mammals, because injection of dsRNA is often used as a control for antisense experiments, albeit at the individual cell (and not organism) level. Nevertheless, perhaps specific 'knockouts' can be generated this way, for organisms in which genetic material cannot be delivered by microinjection. Whatever the mechanism might be, dsRNA-mediated inhibition of gene expression will provide a useful alternative for working out gene function in *C. elegans* and, maybe, in other animals and plants. □

Richard W. Wagner and Lin Sun are at Phylos Inc., 300 Putnam Avenue, Cambridge, Massachusetts 02139, USA.
e-mail: rwagner@phylos.com

1. Schuler, G. D. *et al. Science* 274, 540–546 (1997).
2. Zwaal, R. R., Broeks, A., van Meurs, J., Groenen, J. T. & Plasterk, R. H. *Proc. Natl Acad. Sci. USA* 90, 7431–7435 (1993).
3. Fire, A., Albertson, D., Harrison, S. W. & Moerman, D. G. *Development* 113, 503–514 (1991).
4. Jansen, G., Hazendonk, E., Thijssen, K. L. & Plasterk, R. H. *Nature Genet.* 17, 119–121 (1997).
5. http://www.nemapharm.com
6. Fire, A. *et al. Nature* 391, 806–811 (1998).
7. Bass, B. L. & Weintraub, H. *Cell* 55, 1089–1098 (1988).
8. Wagner, R. W., Smith, J. E., Cooperman, B. S. & Nishikura, K. *Proc. Natl Acad. Sci. USA* 86, 2647–2651 (1989).
9. O'Connell, M. A. *Curr. Biol.* 7, R437–R439 (1997).
10. Maas, S., Melcher, T. & Seeburg, P. H. *Curr. Opin. Cell Biol.* 9, 343–349 (1997).

Liquid crystals

New designs in cholesteric colour

Peter Palffy-Muhoray

Since their discovery in 1888, cholesteric liquid crystals have been subject to considerable attention, resulting in applications in ink and paint technologies, flat-panel displays and thermal imaging. Writing in *Advanced Materials*[1], Tamaoki and co-workers describe a new technique for rewritable full-colour image recording on thin cholesteric films. The low-molecular-weight compound they have developed for this purpose is a cholesteric glass, which is stable at room temperature and which could have applications in optics as well as information display and storage.

The optical properties of cholesterics have made them useful in display[2,3] and laser technologies[4] as well as in the visual arts[2]. In reflected light, cholesterics show intense iridescent colours with a metallic sheen, as seen on scarab beetles. In these materials, rod-like molecules are orientated, on the average, parallel to one another in a given plane, so that the direction of orientation varies linearly with position in the direction normal to the plane. This results in a spatially periodic twisted helical structure as shown in

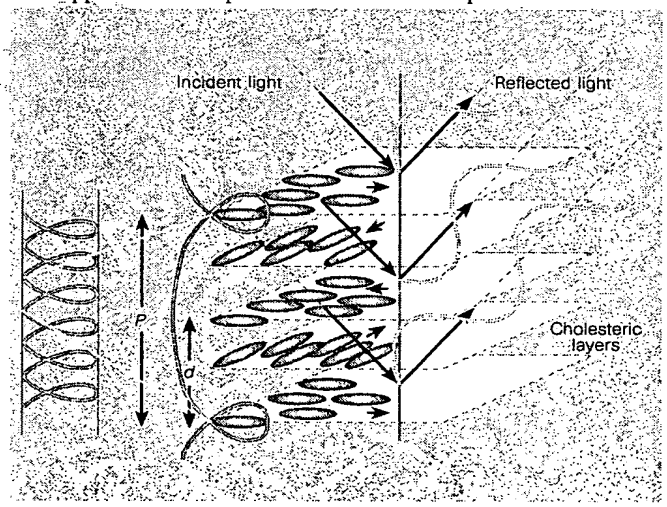

Figure 1 **Sketch of cholesteric structure, showing the dependence of molecular orientation on position. The tip of a vector indicating local molecular orientation traces out a helix. Reflected light waves satisfying the Bragg condition emerge in-phase and add constructively. In the work discussed here, Tamaoki *et al.*[1] have developed a cholesteric glass that is rewritable and stable at room temperature (see Fig. 3).**

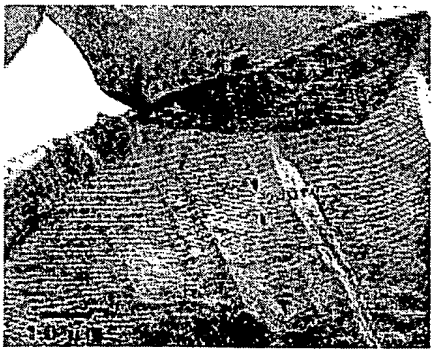

Figure 2 **Transmission electron micrograph of freeze-fractured helical cholesteric. The pitch is 240 nm. (From ref. 12.)**

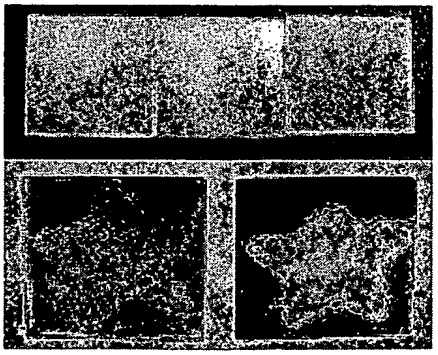

Figure 3 **Photographs of thermally addressed and quenched cholesteric solid films. (From ref. 1.)**